Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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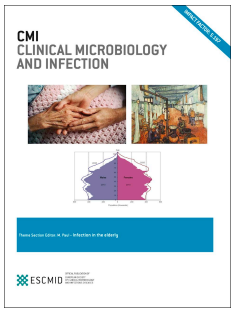

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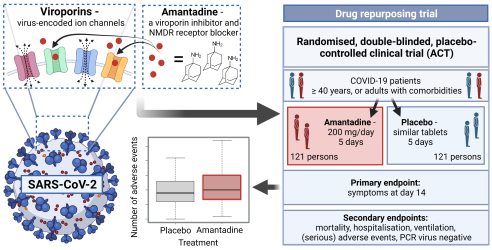

Title

Amantadine for COVID-19 treatment (ACT Study): a randomized, double-blinded, placebo-controlled clinical trial

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ABSTRACT

Objectives

The COVID-19 pandemic has revealed a severe need for effective antiviral treatment. The objectives of this study were to assess if preemptive treatment with amantadine for COVID-19 in non-hospitalized persons ≥40 years or adults with comorbidities was able to prevent disease progression and hospitalization. Primary outcomes were clinical status on day 14.

Methods

Between 9th June 2021 and 27th January 2022, this randomized, double-blinded, placebo-controlled, single-center clinical trial included 242 subjects with a follow-up period of 90 days. Subjects were randomized 1:1 to either amantadine 100 mg or placebo twice daily for five days. The inclusion criteria were confirmed SARS-CoV-2 infection and at least one of (i) age ≥ 40 years, age ≥ 18 years (ii) and at least one comorbidity, or - (iii) and BMI ≥ 30. The study protocol was published at www.clinicaltrials.gov (unique protocol #02032021) and at www.clinicaltrialregister.eu (EudraCT-number 2021-001177-22).

Results

With 121 participants in each arm, we found no difference in the primary endpoint with 82 participants in the amantadine arm, and 92 participants in the placebo arm with no limitations to activities, respectively, and 25 and 37 with limitations to activities in the amantadine arm and the placebo arm respectively. No participants in either group were admitted to hospital or died. The Odds Ratio of having state severity increased by 1 in the amantadine group versus placebo was 1.8 (Confidence Interval 1.0-3.3, (p=0.051)). At day 7, one participant was hospitalized in each group; throughout the study this increased to five and three participants for amantadine versus placebo treatment (P=0.72). Similarly, at day 7, there was no difference in the status of oropharyngeal swabs. Most participants (108 in each group) were SARS-CoV-2 RNA positive (p=0.84).

Conclusions

We found no effect of amantadine on disease progression of SARS-CoV-2 infection.

Keywords

amantadine COVID-19, drug repurposing, ion channels, randomized clinical trial, viroporins

MANUSCRIPT

Introduction

During the pandemic caused by Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2), there has been a severe need for effective antiviral treatment with an impact on long-term morbidity and mortality. The fastest way of identifying such a treatment may be the use of repurposed drugs. Amantadine was initially used for treatment and prophylaxis of influenza A [1], but due to resistance development no longer routinely used [2]. Amantadine, given to patients with Multiple Sclerosis (MS) and Parkinson´s Disease (PD) for improvement of fatigue and treatment of levodopa dyskinesia, respectively, seems to reduce the risk of SARS-CoV-2 infection [3-6]. The underlying mechanism could be that amantadine inhibits replication and/or virulence of SARS-CoV-2 [7, 8] and blocks the ion channel activity of Protein E and ORF10 from SARS-CoV-2 *in vitro* [9, 10]. In support of this, *in vitro* studies have shown that amantadine blocks ion channel activity of Protein E from the closely related SARS-CoV-1 and that this viroporin is central for virus-mediated lung pathology in mice [11-13].

The objective of this study was therefore to assess if preemptive treatment with amantadine against COVID-19 in non-hospitalized adults ≥ 40 years or adults with comorbidities would be able to prevent disease progression and hospitalization.

Methods

Study design

We designed a single center, randomized, double-blinded, placebo-controlled clinical trial of capsule amantadine 100 mg versus capsule placebo (lactose monohydrate), twice daily for five days, in patients with COVID-19 at the Department of Infectious Diseases, Copenhagen University Hospital, Hvidovre, Denmark. The Research Ethics Committee of the Capital Region of Denmark approved the study (J.nr.: H-21021001), and the protocol (Supplementary File 1) was published at www.clinicaltrials.gov (unique protocol #02032021) and at www.clinicaltrialsregister.eu (EudraCT-number 2021-001177-22).

Participants

We identified eligible patients with confirmed SARS-CoV-2 infection through lists of SARS-CoV-2 tested inhabitants in the Capital Region in Denmark identified with nucleic acid positive polymerase chain reaction (PCR) within 5 days prior to inclusion. The lists were once-daily sent electronically from Statens Serum Institut, Copenhagen, Denmark, to the Department of Infectious Diseases, Copenhagen University Hospital, Hvidovre, Denmark, through a secured Virtual Private Network (VPN) system. Candidates, who a-priori fulfilled the inclusion criteria, were subsequently invited electronically by letter with information on the study and contact details through their secured webmail 'e-Boks', which is a trusted provider of secure platforms and digital postboxes in Denmark. Candidates could answer their 'e-Boks'- invitation per e-mail and would then be called by a study nurse or a medical student regarding confirmation of inclusion – and discussion of exclusion - criteria.

Inclusion and exclusion criteria

The population at risk of developing severe COVID-19 was defined through the following *inclusion criteria:* Positive PCR within 5 days prior to inclusion and 1) age ≥ 40 years or age ≥ 18 years and at least one of the following comorbidities: a) chronic heart disease without heart failure or proarrhythmic conditions or ventricular arrhythmias, b) diabetes, c) chronic lung disease, d) hypertension, e) chronic kidney disease GFR<60 ml/minute, f) BMI ≥30 kg/m2, 2) for women of childbearing age (defined as non-sterile

premenopausal women), a negative pregnancy test and willingness to use a contraceptive during the study period (90 days) and 3) signed informed consent. *Exclusion criteria* were: 1) current hospitalisation, 2) allergy to amantadine hydrochloride, rimantadine or inactive ingredients, 3) known history of: a) untreated narrow-angle glaucoma, b) kidney disease, with estimated glomerular filtration rate (eGFR) /1.73m^2 < 35 ml/min, which is calculated based on the patient´s age, sex, race and creatinine level, c) heart failure, proarrhythmic conditions, ventricular arrhythmias, d) seizures, e) Parkinson's Disease, f) gastric ulcer, g) liver disease, h) hereditary galactose intolerance, lactose intolerance or glucose/galactose malabsorption, 4) current use of: a) neuroleptics/antipsychotics/ levodopa, b) anticholinergics, c) thiazides, 5) concurrent malignancy requiring chemotherapy and 6) pregnancy and breastfeeding.

Randomization and masking

All patients, who fulfilled the inclusion criteria, and had none of the exclusion criteria, signed informed consent prior to randomization. Confirmation of study eligibility of the participant was performed by a blinded investigator entering key variables into a secure web-based program Research Electronic Data Capture (REDCap). Unblinded personnel at the regional pharmacy would subsequently use Sealed Envelopes for patient randomization into one of two arms (ratio 1:1). The randomization list was generated centrally in random blocks. All investigators, outcome assessors, and study participants were blinded to the treatment allocation. Unblinding could be performed 24/7. If the treatment of a patient was unblinded, the treatment was discontinued while the patient remained subject to follow up.

Procedures

Active treatment as well as placebo treatment were prepared, packaged, and labeled by pharmacists at The Capital Region Pharmacy, Copenhagen, Denmark. All treatment was delivered in non-transparent identical

capsules. Treatment adherence was assessed through daily web-based questionnaires on study days 2-6 and by collection of the medicine box at assessment on study day 7. Participants randomized to active treatment with amantadine received a daily dose of 200 mg amantadine with 1 capsule (100 mg) two times daily for a total of five days. This dose was based on amantadine use for influenza A treatment/prophylaxis at 200 mg daily for up to 6 weeks. Patients with reduced renal function (an eGFR/1.73m^2 of 35-60 ml/min) received only 100 mg once daily. Participants randomized to placebo treatment received lactose monohydrate oral placebo capsules.

Clinical assessment

At inclusion on study day 1, information regarding age, sex, duration, and status of symptoms of COVID-19, medical history, alcohol, and tobacco use and allergies was obtained via interviews in a mobile clinic at Copenhagen University Hospital, Hvidovre, Denmark, and from medical records. Medical history deemed relevant was SARS-CoV-2 vaccination, liver disease, ischemic heart disease, congestive heart failure, cerebrovascular disease, renal disease, chronic obstructive pulmonary disorder (COPD), diabetes mellitus, neoplastic disease, hematologic disease, peripheral vascular disease, dementia, connective tissue disease and peptic ulcer. Vital signs were assessed, and serum creatinine was measured (for the evaluation of eGFR/1.73m^2). If eGFR/1.73m^2 was above 35 ml/minute the potential participant could be enrolled. Pregnancy test was performed if relevant. Results were entered into REDCap. On study days 2-6, 14, 28 and 90, all study participants completed an online questionnaire in REDCap via a link that was sent to their 'e-Boks' regarding symptoms of COVID-19 and of potential adverse events.

On study day 7 an in-person assessment was performed of the study participant´s symptoms of COVID-19, potential adverse events and on-treatment adherence. Further, an oropharyngeal swab was collected and analyzed for SARS-CoV-2 RNA.

Information regarding admission of a study participant to hospital within the follow up period was obtained from the Danish National Patient Registry at study completion. Reasons for admissions and information on

the use of oxygen, high flow oxygen, mechanical ventilation, and other supportive care was retrieved. All collected biological material was analyzed immediately and subsequently destroyed.

Apart from the participant's own withdrawal of written consent, participants would be withdrawn from the study in case of unintended serious adverse event related to the treatment. No participants were lost to follow up. Protocol violation was reported if participants did not receive the full dose of the study drug (amantadine or placebo) or failed to answer the questionnaires.

Margins were allowed of 24 hours after receiving the questionnaire on study days 2-6 and 14, 72 hours after receiving the questionnaire on study day 28, and of up to 7 days after receiving the questionnaire on study day 90. A reminder was sent within 10 hours if the questionnaire had not been answered. If the questionnaire was still not answered after 23 hours, the patient was contacted by phone.

Outcomes

Primary outcomes were clinical status on day 14, assessed by the ordinal scale (World Health Organization, (WHO)) and categorized into four different patient states: 'Ambulatory' ('No limitations to activities'=1, and 'Limitations to activities'=2); 'Hospitalized mild disease' ('No oxygen therapy'=3, and 'Oxygen by mask or nasal prongs'=4); 'Hospitalized severe disease' ('Non-invasive ventilation or high flow oxygen'=5, 'Intubation and mechanical ventilation'=6, 'Ventilation + additional organ support-pressors, ECMO'=7 and 'Dead' ('Death'=8).

Secondary outcomes were: 1) mortality rate on study day 7, 14, 28 and 90; 2 and 3) incidence of invasive mechanical ventilation (2) or hospitalization (3) at study day 7, 14, 28 and 90; 4) duration of hospitalization; 5) proportion of patients with PCR virus negativity on study day 7, as determined from oropharyngeal swabs; 6) frequency of adverse events; 7) frequency of serious adverse events. Assessment of adverse events and outcome measures were obtained at the assessment on day 7, and from the online questionnaires that were evaluated weekly.

Statistical analyses

A total of 242 study participants with 121 in each treatment arm was chosen to secure a statistical power of 80% to detect an Odds Ratio (OR) of 0.5 for the primary outcome of 14th-day symptom status severity. The primary endpoint was assessed on the described ordinal scale (levels I-VIII) with a proportional odds model, adjusting for sex, age, and vaccination for SARS-CoV-2, where only patients with observed values of all these factors were considered in the analysis. Continuous variables are presented by medians and 25% and 75% quartiles and were analyzed with Mann-Whitney tests. Categorical data are presented as counts with frequencies and were analyzed with chi-square tests if cell counts are above 5, and with Fisher's exact tests if cells are below 5. Analyses were conducted for the full sample of 242 study participants according to the intention-to-treat principle, where treatment assignment is considered equal to treatment. Subsequently the same set of analyses were conducted on a per protocol basis as supplement, where only participants fully following the assigned treatment were considered. The functions glm, chisq.test, fisher.test, prop.test, and wilcox.test from the stats package in R were used for the statistical analyses.

Results

Screening of citizens

Between 9th June 2021 and 27th January 2022, 57,458 citizens were invited via 'e-Boks' to participate in the study of whom 762 were screened for eligibility and 242 were included. (Figure 1).

Study participants

242 participants were enrolled and randomly assigned to receive either amantadine (N=121; 50%) or placebo (N=121; 50%), and all received at least one dose of the study drug. Table 1 shows that baseline demographic characteristics for participants in both groups.

205

206 *Primary Statistics*

207 On study day 14, there was no difference in primary outcome between the two groups (Table 2).
208 The OR, adjusted for sex, age, and vaccination for SARS-CoV-2, of having state severity score increased by 1
209 when receiving amantadine versus placebo was estimated to be 1.8 (Confidence Intervals (CI) 1.0 - 3.3), p-
210 value=0.051, based on a complete case analysis. The sex value was missing for 5 subjects in the Amantadine
211 arm and for 2 subjects in the placebo arm, all of which reported no limitations to activities (score 1).

212 There was no difference between the two groups in hospitalization status counts at study day 7 nor at any
213 time point after entering the study. (Table 3). The number of adverse events per person in each treatment
214 group is shown in Figure 2, with an estimate of the median number of adverse events 1.0 below the median
215 estimate in the placebo group, confidence interval of (-3.0, 0.0) and p value of 0.046. No difference among
216 the numbers of serious adverse events experienced per person was detected in the two groups, p value of
217 0.48. The primary statistics were done as Intention-To-Treat (ITT) analysis and all analyses were repeated as
218 Per Protocol (PP) analysis revealing similar results, though with a non-significant p value in the comparison
219 of adverse events in this case, p value of 0.12 (Supplementary Figure 2A).

220 As supplementary analyses, the main endpoint was analyzed for only the subjects having a comorbidity,
221 both with an ITT analysis (64 subjects) and with a PP analysis (61 subjects). We obtained estimated ORs of
222 1.9 (CI: (0.55, 6.6), p-value 0.32) and 1.8 (CI: (0.53, 5.9), p-value 0.36), respectively. The estimated effect
223 sizes are similar to that of the main analyses, but the uncertainties of the estimates are larger due to the
224 smaller sample size.

225

226 **Discussion**

In this randomized, double-blinded, placebo-controlled, clinical trial, where we investigated the effect of amantadine against COVID-19 in a group of non-hospitalized adults ≥ 40 years or adults with comorbidities, we were not able to demonstrate an effect of preemptive therapy with amantadine versus placebo on our primary outcome of disease progression. Most study participants experienced only relatively few and mild symptoms, making it more difficult to demonstrate any difference in self-reported symptoms between those who received amantadine versus those who received placebo treatment. We were unable to demonstrate an effect of amantadine on hospitalization, as only a few study participants were hospitalized. In addition, none were intubated, underwent mechanical ventilation, received extracorporeal membrane oxygenation (ECMO) or died (Tables 2 and 3). We found no difference in oropharyngeal clearance among individuals treated with amantadine versus placebo consistent with previous studies [14, 15].

A positive effect of amantadine on symptoms caused by SARS-CoV-2 infection has been indicated from *in vitro* and *in silico* studies [7, 9, 16-19], reviews [20, 21], observational studies [22], in patients with MS and PD [3, 4, 23] and in treatment of influenza A [20]. For SARS-CoV-2, amantadine has been shown to block the ion channel activity *in vitro* of the pentameric Protein E, and of ORF10 [9, 16], two viroporins with impact on disease progression (Protein E) and suppression of the innate immune system (Orf10). Viroporins are present in several pathogenic viruses where they contribute to the viral life cycle and have a huge impact on pathogenesis in the host [24]. Therapeutic targeting of these is, however, largely unexplored and at present only exploited clinically in the targeting of M2 from influenza A [25].

In a high throughput drug screen, Smieszek et al. demonstrated amantadine (10uM) to disrupt the lysosomal pathway, hence, interfering with the capacity of the virus to replicate by acting as a lysosomotropic agent [17]. One could argue that the dose of 200 mg orally per day or only five days treatment were insufficient. In vitro, an ID_{50} of 83-119 uM was observed for reduction of virus particles from infected cells [7]. In vivo, the highest tolerable dose of amantadine is 600 mg, resulting in a plasma dose of only 14,6 uM [26]. This supports that a daily dose of 200 mg may be too low.

At initiation of our study, the only launched, available anti-viral and anti-inflammatory combination therapy was intravenous remdesivir for 5 days with oral dexamethasone for 10 days and indicated only for hospitalized COVID-19 patients with oxygen-demand. Even though new oral agents such as molnupiravir and nirmatrelvir have been approved for the treatment of pre-hospital SARS-CoV-2 infection since then [27], these treatment options have only moderate efficacy and considerable interactions, respectively, and are not available on a global level.

This study had limitations. Firstly, the procedure with an invitation through 'e-Boks' delayed inclusion in relation to symptom onset. In theory, possible symptoms could already have been subsiding spontaneously. Secondly, there might have been selection bias of participants who were not suffering severe symptoms. Thirdly, we treated participants with only 200 mg amantadine orally per day in only five days which might have been an insufficient dose/dosing period. Fourthly, data regarding symptoms and adherence relied on the participants' self-reported symptoms. Fifthly, different variants dominated the SARS-CoV-2 pandemic, causing fewer symptoms than the previous variants, thus maybe making it difficult to show an effect of amantadine versus placebo.

In conclusion, we were not able to demonstrate any effect of amantadine versus placebo on disease progression of SARS-CoV-2 infection. These results are important so that future COVID-19 individuals are not treated with amantadine based on a belief of a positive effect from *in vitro* and non-randomized data.

Author contribution Statement

MMR and TNK conceptualized the idea to this study and obtained the necessary funding. NW administered the clinical project and participated in the investigation of study participants together with SB, JDS, JBG, CV and LRJ. NW wrote the first draft of the report with input from SB and MMR. CS did the statistical analysis. All authors had full access to all the data in the study, read the manuscript critically and had final

responsibility for the decision to submit for publication. NW, SB, and LRJ have directly accessed and verified the underlying data reported in the manuscript.

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The Bio Innovation Institute (BII), Copenhagen, Denmark, supported the study financially and had no role in study design, patient recruitment, in the collection, analysis, or interpretation of data, in the writing of the report, in the decision to submit the paper for publication or any aspect pertinent to the study. None of the authors have been paid to write this article by a pharmaceutical company or other agency.

Competing interests

NW has been Clinical Investigator for Abbvie, MSD, and has received unrestricted grants for research from Abbvie, Gilead; all payments made to her institution. TK is founder, CEO, and a minority shareholder of Synklino A/S. MMR is founder and a minority shareholder of Synklino A/S. SB; JDS; JBG; CV; LRJ and CS have no competing interests.

Figure Legends

Figure 1: Trial profile of the study population in the ACT Study.

Figure 2: Boxplots of the number of adverse events experienced per person in the two treatment groups after starting the study. The length of the box represents the interquartile range (25th–75th percentiles); the horizontal line inside the box represents the median; and the vertical lines issuing from the box extend to the minimum and maximum values.

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Table 1. Demographic characteristics, symptoms, and comorbidity for the intention-to-treat study population of N=242 individuals randomized to amantadine (n=121) or placebo (n=121) for five days.

Variable	Level	Placebo (n=121)	Amantadine (n=121)
Compliance	0	9 (7.4)	7 (5.8)
	1	112 (92.6)	114 (94.2)
Sex	Female	57 (47.9)	53 (45.7)
	Male	62 (52.1)	63 (54.3)
	missing	2	5
Age (years)	median [iqr]	50.5 [43.5, 56.9]	50.9 [45.1, 58.3]
BMI (kg/m^2)	median [iqr]	27.4 [23.8, 30.8]	26.5 [23.8, 30.4]
Alcohol per week (units)	median [iqr]	2 [1,6]	3 [1,8]
Smoking	Never	63 (52.1)	62 (51.2)
	Previously	49 (40.5)	51 (42.1)
	Actively	9 (7.4)	8 (6.6)
Vaccinated	No	1 (0.8)	1 (0.8)
	Yes	120 (99.2)	120 (99.2)
Days since vaccinated	median [iqr]	333 [306.5, 362.0]	332.5 [303.5, 362.2]
	missing	6	5
Symptoms	No	1 (0.8)	3 (2.5)
	Yes	120 (99.2)	118 (97.5)
Headache	No	47 (38.8)	50 (41.3)
	Yes	74 (61.2)	71 (58.7)
Cough	No	18 (14.9)	28 (23.1)
	Yes	103 (85.1)	93 (76.9)
Throat pain	No	74 (61.2)	71 (58.7)
	Yes	47 (38.8)	50 (41.3)
Fever	No	68 (56.2)	74 (61.2)
	Yes	53 (43.8)	47 (38.8)
Respiratory distress	No	80 (66.1)	83 (68.6)
	Yes	41 (33.9)	38 (31.4)
Muscle soreness	No	75 (62.0)	66 (54.5)
	Yes	46 (38.0)	55 (45.5)
Exhaustion	No	31 (25.6)	29 (24.0)
	Yes	90 (74.4)	92 (76.0)
Cold symptoms	No	33 (27.3)	30 (24.8)
	Yes	88 (72.7)	91 (75.2)
Joint pain	No	87 (71.9)	92 (76.0)
	Yes	34 (28.1)	29 (24.0)
Chest pain	No	98 (81.0)	100 (82.6)
	Yes	23 (19.0)	21 (17.4)
Vomiting	No	116 (95.9)	118 (97.5)
	Yes	5 (4.1)	3 (2.5)
Diarrhea	No	109 (90.1)	112 (92.6)
	Yes	12 (9.9)	9 (7.4)
Changed taste	No	75 (62.0)	73 (60.3)

	Yes	46 (38.0)	48 (39.7)
Changed smell	No	72 (59.5)	75 (62.0)
	Yes	49 (40.5)	46 (38.0)
Others	No	87 (71.9)	92 (76.0)
	Yes	34 (28.1)	29 (24.0)
Comorbidity	No	83 (68.6)	95 (78.5)
	Yes	38 (31.4)	26 (21.5)
Dementia	No	121 (100.0)	121 (100.0)
	Yes	0 (0.0)	0 (0.0)
Lung disease	No	107 (88.4)	108 (89.3)
	Yes	14 (11.6)	13 (10.7)
Rheumatic disease	No	105 (86.8)	109 (90.1)
	Yes	16 (13.2)	12 (9.9)
Diabetes Mellitus	No	115 (95.0)	119 (98.3)
	Yes	6 (5.0)	2 (1.7)
Hemi paraplegia	No	119 (98.3)	120 (99.2)
	Yes	2 (1.7)	1 (0.8)
Kidney disease	No	120 (99.2)	117 (96.7)
	Yes	1 (0.8)	4 (3.3)
HIV	No	119 (98.3)	121 (100.0)
	Yes	2 (1.7)	0 (0.0)
Hematological disease	No	120 (99.2)	121 (100.0)
	Yes	1 (0.8)	0 (0.0)
Cancer	No	117 (96.7)	117 (96.7)
	Yes	4 (3.3)	4 (3.3)

Table 2. Primary outcomes on study day 14: clinical status assessed by the ordinal scale suggested by WHO[¤] and categorized into four different patient states*				
Patient state	Descriptor	Score	Placebo group Number of study participants	Amantadine group Number of study participants
1.Ambulatory	No limitations to activities	I	94	82
	Limitations to activities	II	25	37
2.Hospitalized mild disease	Hospitalized no oxygen therapy	III	0	0
	Oxygen by mask or nasal prongs	IV	0	0
3.Hospitalized severe disease	Non-invasive ventilation or high flow oxygen	V	0	0
	Intubation and mechanical ventilation	VI	0	0
	Ventilation + additional organ support -pressors, ECMO	VII	0	0
4.Dead	Death	VIII	0	0

[¤]WHO: The World Health Organization.

*Data missing for 2 patients in the amantadine – and the placebo group, respectively.

[#] ECMO: Extra Corporal Membrane Oxygenation.

Table 3. Secondary outcomes obtained at the assessment on study day 7, from the online questionnaires at study day 14, 28, and 90, and from medical records.

	Placebo group N = 121	Amantadine group N = 121
Mortality rate during the study	0	0
Patients with incidence of invasive mechanical ventilation during the study	0	0
Patients hospitalized during the study	3	5
Total duration of hospitalization in days	20	20
Number of patients with PCR virus negativity on study day 7, as determined from oropharyngeal swabs[¤]	10	11
Median number of adverse events per person (range)	9 (6-13)	10 (7-15)
Median number of serious adverse events per person	0	0

[¤]108 study participants were PCR-positive, and 3 - and 2 study participants did not have a swab taken, in the placebo- and amantadine-groups, respectively.

CONSORT 2010 Flow Diagram